Use of LysoTracker to detect programmed cell death in embryos and differentiating embryonic stem cells.

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Authors: Jennifer L Fogel, Thu Zan Tun Thein, Francesca V Mariani

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Public Summary:
Programmed cell death (PCD) occurs in adults to maintain normal tissue homeostasis and during embryological development to shape tissues and organs. During development, toxic chemicals or genetic alterations can cause an increase in PCD or change PCD patterns resulting in developmental abnormalities and birth defects. To understand the cause of these defects, the study of embryos can be complemented with in vitro assays that use differentiating embryonic stem (ES) cells. Here we describe a simple protocol using highly soluble and fixable dye called LysoTracker to look at PCD in mouse embryos null for the growth factor, sonic hedgehog. In addition, we demonstrate how to analyze PCD using LysoTracker in differentiating ES cell cultures. In summary, LysoTracker staining can be a great complement to other methods of detecting PCD in tissues and differentiating ES cells.

Scientific Abstract:
Programmed cell death (PCD) occurs in adults to maintain normal tissue homeostasis and during embryological development to shape tissues and organs(1,2,6,7). During development, toxic chemicals or genetic alterations can cause an increase in PCD or change PCD patterns resulting in developmental abnormalities and birth defects(3-5). To understand the etiology of these defects, the study of embryos can be complemented with in vitro assays that use differentiating embryonic stem (ES) cells. Apoptosis is a well-studied form of PCD that involves both intrinsic and extrinsic signaling to activate the caspase enzyme cascade. Characteristic cell changes include membrane blebbing, nuclear shrinking, and DNA fragmentation. Other forms of PCD do not involve caspase activation and may be the end-result of prolonged autophagy. Regardless of the PCD pathway, dying cells need to be removed. In adults, the immune cells perform this function, while in embryos, where the immune system has not yet developed, removal occurs by an alternative mechanism. This mechanism involves neighboring cells (called "non-professional phagocytes") taking on a phagocytic role-they recognize the 'eat me' signal on the surface of the dying cell and engulf it(8-10). After engulfment, the debris is brought to the lysosome for degradation. Thus regardless of PCD mechanism, an increase in lysosomal activity can be correlated with increased cell death. To study PCD, a simple assay to visualize lysosomes in thick tissues and multilayer differentiating cultures can be useful. LysoTracker dye is a highly soluble small molecule that is retained in acidic subcellular compartments such as the lysosome(11-13). The dye is taken up by diffusion and through the circulation. Since penetration is not a hindrance, visualization of PCD in thick tissues and multi-layer cultures is possible(12,13). In contrast, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) analysis(14), is limited to small samples, histological sections, and monolayer cultures because the procedure requires the entry/permeability of a terminal transferase. In contrast to Aniline blue, which diffuses and is dissolved by solvents, LysoTracker Red DND-99 is fixable, bright, and stable. Staining can be visualized with standard fluorescent or confocal microscopy in whole-mount or section using aqueous or solvent-based mounting media(12,13). Here we describe protocols using this dye to look at PCD in normal and sonic hedgehog null mouse embryos. In addition, we demonstrate analysis of PCD in differentiating ES cell cultures and present a simple quantification method. In summary, LysoTracker staining can be a great complement to other methods of detecting PCD.